

We claim:

1. A composition for use in targeting endothelial cells, tumor cells or other cells which express NP-1, which comprises a compound of the formula (I)



5 in which

A is a monomer, multimer or polymer of TKPPR (SEQ ID NO: 2), or a TKPPR (SEQ ID NO: 2) analogue which specifically binds to NP-1 or cells that express NP-1 with avidity that is equal to or greater than TKPPR (SEQ ID NO: 2);

10 L is a linker; and

B is a substrate.

2. A composition according to claim 1, wherein A is a multimer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue.

3. A composition according to claim 2, wherein A is a tetramer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue.

- 20 4. A composition according to claim 1, wherein B comprises B<sub>1</sub>, a lipid able to bind the linker in a covalent or non-covalent manner.

5. A composition according to claim 4, in which B<sub>1</sub> comprises a synthetic or naturally-occurring generally amphipathic and biocompatible compound, selected from the group consisting of fatty acids; lysolipids; phospholipids; phosphatidylinositol; sphingolipids; glycolipids; glucolipids; sulfatides; glycosphingolipids; phosphatidic acids; lipids bearing polymers; lipids bearing sulfonated mono- di-, oligo- or polysaccharides; cholesterol, cholesterol sulfate; cholesterol hemisuccinate; tocopherol hemisuccinate; lipids with ether and ester-linked fatty acids; polymerized lipids; diacetyl phosphate; dicetyl phosphate; stearylamine; cardiolipin; phospholipids with short chain fatty acids of about 6 to about 8 carbons in length; synthetic phospholipids with asymmetric acyl chains; ceramides; non-ionic liposomes; sterol esters of sugar acids; esters of sugars and aliphatic acids; saponins; glycerol dilaurate; glycerol trilaurate; glycerol dipalmitate; glycerol; glycerol esters; long chain alcohols; 6-(5-cholesten-3 $\beta$ -yloxy)-1-thio- $\beta$ -D-galactopyranoside; digalactosyl-diglyceride; 6-(5-cholesten-3 $\beta$ -yloxy)hexyl-6-amino-6-deoxy-1-thio- $\beta$ -D-galacto-

pyranoside; 6-(5-cholesten-3 $\beta$ -yloxy)hexyl-6-amino-6-deoxyl-1-thio- $\beta$ -D-manno-pyranoside; 12-(((7'-diethylaminocoumarin-3-yl)carbonyl)methylamino)octadecanoic acid; N-[12-(((7'-diethylaminocoumarin-3-yl)carbonyl)methylamino)octadecanoyl]-2-aminopalmitic acid; N-succinyldioleoylphosphatidylethanolamine; 1,2-dioleoyl-sn-glycerol; 1,2-dipalmitoyl-sn-3-succinylglycerol; 1,3-dipalmitoyl-2-succinylglycerol; 1-hexadecyl-2-palmitoylglycerophosphoethanolamine; palmitoylhomocysteine, and combinations thereof.

6. A composition according to claim 1, wherein B comprises

B<sub>2</sub>, a non-lipid polymer able to bind the linker in a covalent manner.

7. A composition according to claim 6, in which B<sub>2</sub> comprises B<sub>2a</sub> a polymer useful for producing microparticles, or B<sub>2b</sub>, a non-ionic surfactant.

8. A composition according to claim 7 in which B<sub>2a</sub> is selected from the group consisting of polyvinyl alcohol (PVA) and a polyoxyethylene-polyoxypropylene block copolymer.

9. A composition according to claim 7, in which B<sub>2a</sub> comprises a bead which is derivatizable and is attached to a detectable label.

10. A composition according to claim 9, in which the detectable label is a fluorescent or radioactive marker.

11. A composition according to claim 1, in which B comprises a bioactive agent.

12. A composition according to claim 1, in which B comprises a delivery vehicle for genetic material.

13. A composition according to claim 1, in which B comprises a delivery vehicle for a drug or therapeutic.

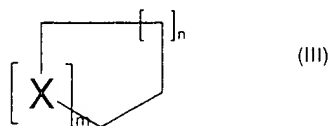
14. A composition according to claim 1, in which B comprises B<sub>c</sub>, a metal chelating group.

15. A composition according to claim 14, in which the metal chelating group is complexed with a metal.

16. A composition according to claim 15, in which the metal chelating group is complexed with a radioactive metal.

17. A composition according to claim 16, in which the metal chelating group is complexed with a radioactive metal useful for radiotherapy.

18. A composition according to claim 16, in which the metal chelating group is complexed with a radioactive metal useful for imaging.
- 5 19. A composition according to claim 16, in which the metal is selected from the group consisting of:  $^{99m}\text{Tc}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{111}\text{In}$ ,  $^{88}\text{Y}$ ,  $^{90}\text{Y}$ ,  $^{105}\text{Rh}$ ,  $^{153}\text{Sm}$ ,  $^{166}\text{Ho}$ ,  $^{165}\text{Dy}$ ,  $^{177}\text{Lu}$ ,  $^{64}\text{Cu}$ ,  $^{97}\text{Ru}$ ,  $^{103}\text{Ru}$ ,  $^{186}\text{Re}$ , and  $^{188}\text{Re}$ .
- 10 20. A composition according to claim 14, in which the metal chelating group Bc is selected from the list consisting of:  $\text{N}_4$ ,  $\text{S}_4$ ,  $\text{N}_3\text{S}$ ,  $\text{N}_2\text{S}_2$  and  $\text{NS}_3$  chelators.
21. A composition according to claim 20, in which the metal chelating group Bc comprises oxa-PnAO.
- 15 22. A composition according to claim 21, in which A comprises a tetramer of TKPPR (SEQ ID NO: 2) and the metal chelating group is complexed to  $^{99m}\text{Tc}$ .
23. A composition according to claim 1, in which L is a bond or is derived from :
- 20 an alkyl chain  $\text{C}_1\text{-C}_{5000}$ , linear or branched, saturated or unsaturated, optionally interrupted or substituted by one or more groups such as: O, S, NR, OR, SR, COR, COOH, COOR, CONHR, CSNHR,  $\text{C=O}$ ,  $\text{S=O}$ ,  $\text{S(=O)}_2$ ,  $\text{P=O(O)}_2\text{OR}$ ,  $\text{P(O)}_2(\text{OR})_2$ , halogens, or phenyl groups, optionally substituted by one or more -NHR, -OR, -SR, -COR, -CONHR, -N-C=S, -N-C=O, halogens, in which
- 25 R is H or an alkyl group  $\text{C}_1\text{-C}_4$ , linear or branched, optionally substituted by one or more -OH;
- such a chain can be interrupted or substituted by one or more cyclic groups  $\text{C}_3\text{-C}_9$ , saturated or unsaturated, optionally interrupted by one or more O, S or NR; by one or more groups such as: -NHR, -OR, -SR, -COR, -CONHR, or a phenyl group optionally
- 30 substituted by one or more -NHR, -OR, -SR, -COR, -CONHR, -N-C=S, -N-C=O, halogens.
24. A composition according to claim 23, in which the cyclic groups present in L are saturated or unsaturated, and correspond to the following general formula (III)



- 35 in which
- n can range from 0 to 4;
- m can range from 0 to 2;
- X can be NH, NR, O, S or SR.

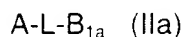
25. A composition according to claim 23, in which the linker L is an oligopeptide comprising 1 to 100 natural or synthetic amino acids.

26. A composition according to claim 25, in which the amino acids are selected from the group consisting of glycine, glutamic acid, aspartic acid,  $\gamma$ -amino-butyric acid and trans-4-aminomethyl-cyclohexane carboxylic acid.

27. A composition according to claim 23, in which L is derived from difunctional PEG- (polyethyleneglycol) derivatives.

28. A composition according to claim 23, in which L is selected from the group consisting of: glutaric acid, succinic acid, malonic acid, oxalic acid and PEG derivatized with two  $\text{CH}_2\text{CO}$  groups.

29. A compound of the formula (IIa) for use in targeting endothelial cells, tumor cells or other cells which express NP-1

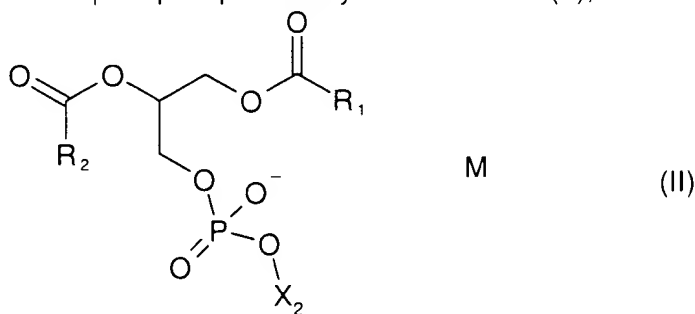


in which

A is a monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue which specifically binds to NP-1 or cells that express NP-1 with avidity that is equal to or greater than TKPPR (SEQ ID NO: 2);

L is a linker; and

$\text{B}_{1a}$  comprises a phospholipid moiety of the formula (II),

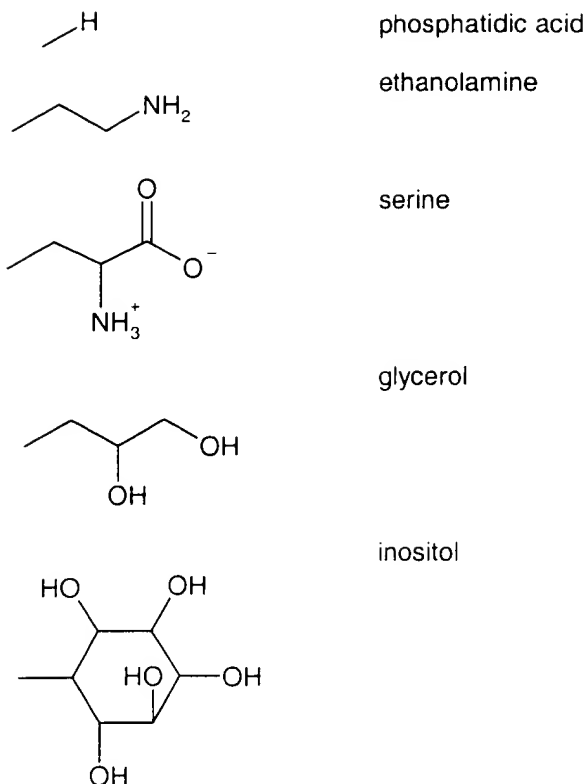


where

M is an alkaline or alkaline- earth metal cation;

$\text{R}_1$  and  $\text{R}_2$  independently, correspond to a linear long chain  $\text{C}_{12}\text{-C}_{20}$ ; saturated or unsaturated, optionally interrupted by  $\text{C}=\text{O}$ , or  $\text{O}$ ; and

$\text{X}_2$  is selected in a group consisting of



30. A compound according to claim 29, in which  $R_1$  and  $R_2$  are independently a saturated linear long chain  $C_{12}-C_{20}$ .

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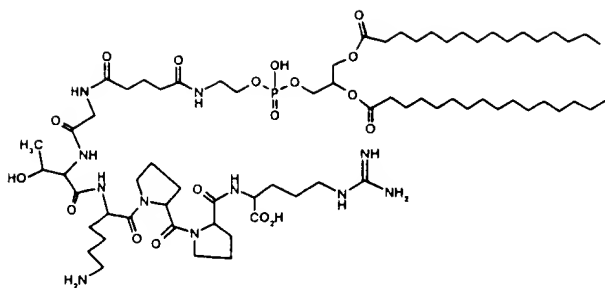
31. A compound according to claim 30, in which the phospholipid of formula (II) comprises a phospholipid selected from the group consisting of: dimyristoylphosphatidylethanolamine, dipalmitoylphosphatidylethanolamine, distearoylphosphatidylethanolamine, diarachidoylphosphatidylethanolamine, dioleoylphosphatidylethanolamine, dilinoleylphosphatidylethanolamine, fluorinated analogues of any of the foregoing, and mixtures of any of the foregoing.

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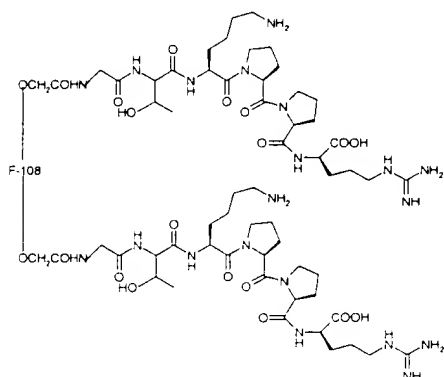
32. A compound according to claim 31, in which the phospholipid of formula (II) comprises dipalmitoylphosphatidylethanolamine.

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33. A composition for use in targeting endothelial cells, tumor cells or other cells which express NP-1, comprising a compound selected from the group consisting of:



and



34. An ultrasound contrast agent comprising a suspension of gas-filled microbubbles, in which the microbubbles comprise a compound of any one of claims 29 to 32.

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35. An ultrasound contrast agent comprising a suspension of gas-filled microbubbles, in which the microbubbles comprise a compound of claim 29 and the gas comprises a fluorinated gas.

10 36. An ultrasound contrast agent comprising a suspension of gas-filled microbubbles in which the microbubbles comprise a compound of claim 29 in which A is TKPPR tetramer and the gas comprises SF<sub>6</sub> or a perfluorocarbon selected from the group consisting of C<sub>3</sub>F<sub>8</sub>, C<sub>4</sub>F<sub>8</sub>, C<sub>4</sub>F<sub>10</sub>, C<sub>5</sub>F<sub>12</sub>, C<sub>6</sub>F<sub>12</sub>, C<sub>7</sub>F<sub>14</sub> and C<sub>8</sub>F<sub>18</sub>.

15 37. A compound for use in targeting endothelial cells, tumor cells or other cells that express NP-1 of the formula



where

20 A is a monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue which specifically binds to NP-1 or cells that express NP-1 with avidity that is equal to or greater than TKPPR (SEQ ID NO: 2);

L is a linker; and

B<sub>3</sub> is a biodegradable, physiologically acceptable polymer.

38. An ultrasound contrast agent comprising a suspension of gas-filled microballoons, in which the microballoons comprise a compound of claim 37.

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39. An ultrasound contrast agent comprising a suspension of gas-filled microballoons, in which the microballoons comprise a compound of claim 37 in which A is a TKPPR tetramer and the gas comprises a gas selected from the group consisting of: air; nitrogen; oxygen; CO<sub>2</sub>; argon; xenon or krypton, a fluorinated gas, a low molecular weight hydrocarbon, an alkene or an alkyne and mixtures thereof.

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40. A compound for use in targeting endothelial cells, tumor cells or other cells which express NP-1 comprising a monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue which specifically binds to NP-1 or cells that express NP-1 with avidity that is equal to or greater than TKPPR (SEQ ID NO: 2).

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41. A compound for use in inhibiting angiogenesis comprising a monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue which specifically binds to NP-1 or cells that express NP-1 with avidity that is equal to or greater than TKPPR (SEQ ID NO: 2).

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42. A pharmaceutical composition for use in targeting endothelial cells, tumor cells or other cells which express NP-1, comprising:

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a monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue which specifically binds to NP-1 or cells that express NP-1 with avidity that is equal to or greater than TKPPR (SEQ ID NO: 2); and

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a pharmaceutically acceptable carrier.

43. A pharmaceutical composition for use in inhibiting angiogenesis comprising:

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a monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue which specifically binds to NP-1 or cells that express NP-1 with avidity that is equal to or greater than TKPPR (SEQ ID NO: 2); and

a pharmaceutically acceptable carrier.

44. A pharmaceutical composition for use in inhibiting angiogenesis comprising:

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a tetramer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue which specifically binds to NP-1 or cells that express NP-1 with avidity that is equal to or greater than TKPPR (SEQ ID NO: 2); and

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a pharmaceutically acceptable carrier.

45. A process for preparing a compound of ~~claim 1~~ comprising:

a) obtaining a monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) or an analogue thereof;

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b) conjugating the monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) with the linker to give a compound of formula (IIb) ; and

A-L (IIb)

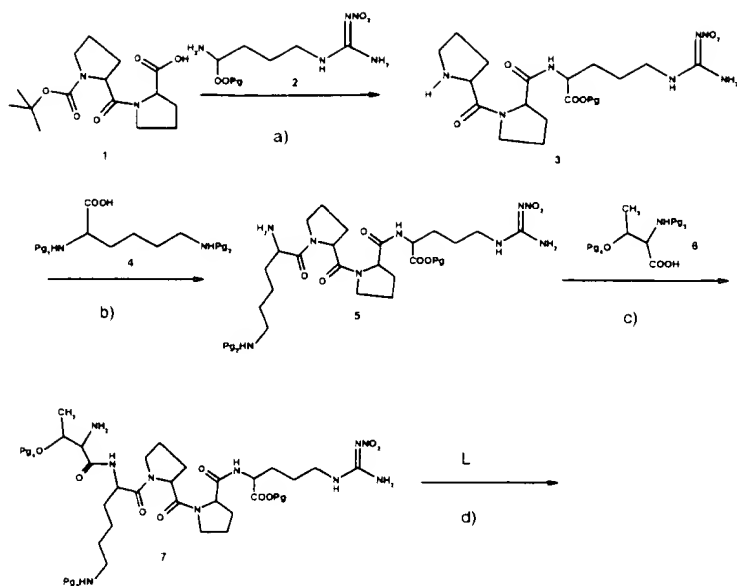
c) forming a covalent or non-covalent bond between a compound of formula (IIb) and the substrate B or forming a covalent bond between the substrate B and the linker to form a conjugate B-L, and

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conjugating of the conjugate B-L with the monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) or an analogue thereof.

46. A process according to ~~claim 45~~, in which the compounds of formula (IIb) are prepared as illustrated in the following Scheme

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(Pg = protecting group)

in which

the steps a), b), and c) are all condensation reactions performed under basic conditions, and step d) is a condensation in basic conditions with the linker.

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47. A method of imaging an angiogenic site in an human or animal comprising:

a) administering to said human or animal a composition comprising a compound of the formula (I)

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A-L-B (I)

in which

A is a monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue which specifically binds to NP-1 or cells which express NP-1 with avidity that is equal to or greater than TKPPR (SEQ ID NO: 2);

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L is a linker; and

B is a substrate, where B comprises a detectable moiety; and

b) detecting said moiety.

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48. A method of imaging endothelial cells, tumor cells or other cells that express NP-1 in a human or animal comprising:

- a) administering to said human or animal a composition comprising a compound of the formula (I)



in which

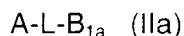
5     A     is a monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue which specifically binds to NP-1 or cells which express NP-1 with avidity that is equal to or greater than TKPPR (SEQ ID NO: 2);

L     is a linker; and

10    B     is a substrate, where B comprises a detectable moiety; and

b) detecting said moiety.

49. A method of ultrasound imaging comprising administering an ultrasound contrast agent comprising a suspension of gas-filled microbubbles, in which the microbubbles comprise a compound of the formula (IIa)

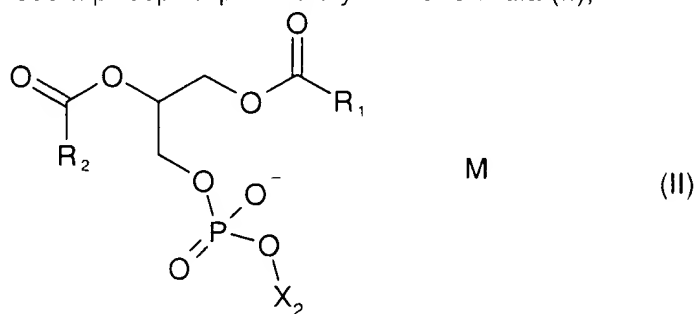


in which

20    A     is a monomer, multimer or polymer of TKPPR (SEQ ID NO: 2) or a TKPPR (SEQ ID NO: 2) analogue which specifically binds to NP-1 or cells which express NP-1 with avidity that is equal to or greater than TKPPR (SEQ ID NO: 2);

L     is a linker; and

B<sub>1a</sub> comprises a phospholipid moiety of the formula (II),



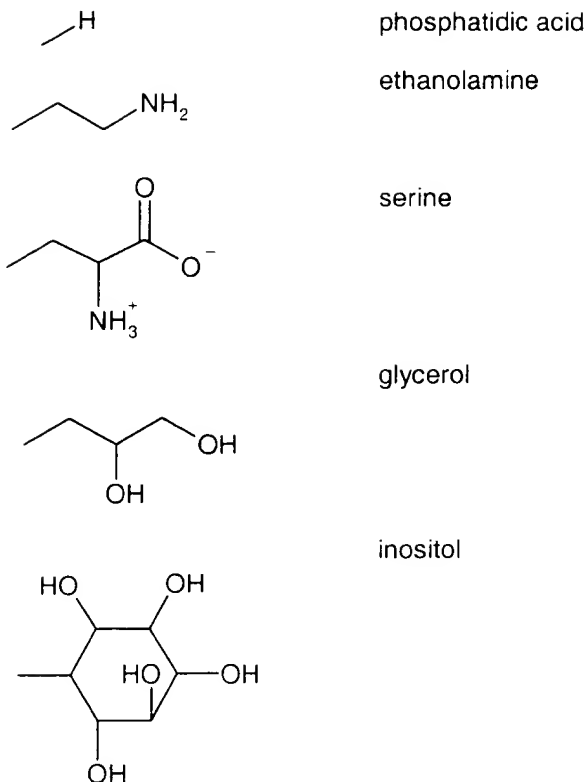
25    where

M     is an alkaline or alkaline- earth metal cation;

R<sub>1</sub> and R<sub>2</sub>     independently, correspond to a linear long chain C<sub>12</sub>-C<sub>20</sub>; saturated or unsaturated, optionally interrupted by C=O, or O; and

X<sub>2</sub>     is selected in a group consisting of

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50. A method of staging a tumor in a human or an animal comprising administering a composition comprising a detectable moiety and a compound of claim 1 to said human or animal and detecting said moiety in said human or animal.

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51. A method of screening at least one agent for the ability of said agent to target endothelial cells, tumor cells or other cells that express NP-1, comprising contacting said cells in vitro with a composition of any one of claims 7 to 9.

10 52. A method of screening at least one targeted ultrasound contrast agent for the ability of said agent to target endothelial cells, tumor cells or other cells that express NP-1, comprising contacting said cells in vitro with a composition of any one of claims 7 to 9.

15 53. A method for the therapeutic delivery in vivo of a bioactive agent to a patient suffering from effects associated with angiogenesis-related disorders comprising administering a therapeutically effective amount of a composition of any one of claims 11 to 13.

54. A method of treating an individual exhibiting effects of an angiogenesis-related disorder comprising administering a therapeutically effective amount of a composition of any one of claims 11 to 13.
- 5 55. A composition according to claim 12, wherein B comprises a delivery vehicle for genetic material selected from the group consisting of: a virus particle, a viral or retroviral gene therapy vector, a liposome, a complex of cationic lipids and genetic material and a complex of dextran derivatives and genetic material.
- 10 56. A method for delivering desired nucleic acids to endothelial cells, tumor cells or other cells expressing NP-1, comprising administering a therapeutically effective amount of the composition of claim 55.
- 15 57. A method of enhancing endothelial cell-targeted gene therapy comprising incorporating compounds of claim 40 in or on the delivery vehicle for genetic material.
58. A method of enhancing tumor cell-targeted gene therapy comprising incorporating compounds of claim 40 in or on the delivery vehicle for genetic material.
- 20 59. A method of enhancing gene therapy targeting angiogenic cells comprising incorporating compounds of claim 41 in or on the delivery vehicle for genetic material.
- 25 60. A method for imaging of a human or animal comprising:
- a) administering to said human or animal a composition according to any one of claims 16, 18, 19, 21 or 22; and
- b) imaging all or part of said human or animal using a camera that detects radiation.
- 30 61. A method for imaging of a human or animal comprising:
- a) administering to said human or animal a composition according to claim 21; and
- b) imaging all or part of said human or animal using a camera that detects radiation.
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62. A method for treating a human or animal with a tumor or angiogenesis-related disease comprising administering to said human or animal a therapeutically effective amount of a composition according to either one of claims 17 or 19.
- 5 63. A kit for preparing a radiopharmaceutical comprising a composition of claim 14 or a pharmaceutically acceptable salt thereof.
64. A kit according to claim 63, further comprising an exchange ligand.
- 10 65. A kit according to either claim 63 or 64, further comprising a reducing agent.